Statistical Analysis Plan

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Trial Statistician	Elham Sabri
Trial Sponsor	Northern Therapeutics, Inc.
SAP Author	Elham Sabri

Revision Control

Protocol Version	Updated SAP version number	Section number changed	Description of change	Date changed

SAP Signatures

SAP Version Number being approved: Version 1.0 08/09/2020

I give my approval for the attached SAP entitled SAPPHIRE dated 08/09/2020.

Trial Statistician

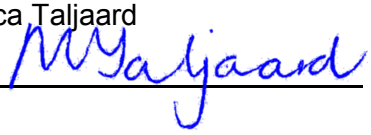
Name: Elham Sabri

Signature: 

Date: 07Oct2020

Senior Statistician

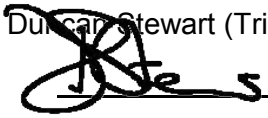
Name: Dr. Monica Taljaard

Signature: 

Date: 07 Oct 2020

Trial Sponsor – Northern Therapeutics, Inc.

Name: Dr. Duncan Stewart (Trial Medical Consultant)

Signature: 

Date: October 7, 2020

Roles and responsibilities

Name	Role	Institution
Elham Sabri	Trial Statistician	Ottawa Hospital Research Institute - Ottawa Methods Centre
Dr. Duncan Stewart	Trial Medical Consultant	Trial Sponsor – Northern Therapeutics, Inc.
Dr. Monica Taljaard	Senior Statistician	Ottawa Hospital Research Institute - Ottawa Methods Centre

Contributions

Elham Sabri developed the statistical analysis plan (SAP) based on the outlined analyses set out in the trial protocol. Dr. Monica Taljaard and Dr. Duncan Stewart reviewed and approved the SAP.

Abbreviations and Definitions

6MWD	6 minute walk distance
AE	Adverse Event
BNP	Brain natriuretic peptide
CAD	Coronary artery disease
CRP	C-reactive protein
DMS	Data management services
DSMB	Data Safety and Monitoring Board
eCRFs	electronic CRFs
EDCS	electronic data capture system
eNOS	endothelial nitric oxide synthase
EPCs	Endothelial progenitor cells
FEV1	Forced expiratory volume at one second
LAVI	Left atrial volume index
MCT	monocrotaline
mPAP	mean pulmonary arterial pressure
MRI	Magnetic Resonance Imaging
NO	Nitric oxide
OMC	Ottawa Methods Centre
PAH	Pulmonary Arterial Hypertension
PCWP	Pulmonary capillary wedge pressure
PHACeT	Pulmonary Hypertension and Cell Therapy
PIPEDA	Personal Information Protection and Electronic Documents Act
PVR	Pulmonary vascular resistance
REB	Research Ethics Board
REML	Restricted Maximum Likelihood
RHC	Right heart cardiac catheterization
TLC	Total lung capacity
VQ	Ventilation and perfusion

Section 1: Introduction

Background and Rationale

Pulmonary Arterial Hypertension (PAH) is a progressive and incurable disease which has a prognosis that is as poor as for many malignancies. PAH is associated with profound perturbations in endothelial function, characterized by an imbalance in the production of endothelium-derived vasoconstrictor and vasodilator factors. In particular, decreased nitric oxide (NO) bioavailability results in inappropriate vasoconstriction, increased muscularization and reduced thromboprotection in the pulmonary circulation. Although there have been a number of advances in the therapy of PAH in the last several decades, all current treatments are essentially palliative and most patients ultimately progress, either receiving lung transplantation as a final resort or succumb to right heart failure. Thus, there is an unmet need for new therapies of PAH that can restore the pulmonary vascular structure and function, and improve pulmonary hemodynamics in advanced PAH.

PAH is characterized by marked increases in pulmonary vascular resistance (PVR). In the early stages of the disease this is thought to be due to arteriolar vasoconstriction and progressive increases in muscularization, particularly of the distal arteriolar bed. However, in more advanced disease, PAH is characterized by widespread loss of pulmonary microvasculature which results either from complex remodeling leading to obliteration or degeneration of pre-capillary arterioles. While the mechanism of lung arteriolar loss is uncertain, there is general consensus that it is triggered by endothelial cell apoptosis, which possibly leads directly to microvascular regression or secondarily to the emergence of growth-dysregulated vascular cells which contribute to luminal occlusion.

We have developed a novel method for achieving selective gene transfer to the pre-capillary arteriolar bed, which is the critical site of pathology in PAH. This method involves the ex vivo transfection of autologous early outgrowth endothelial progenitor cells (EPCs), harvested by apheresis from the patient's own circulating blood, and the introduction of these genetically engineered cells back into the pulmonary circulation through an intravenous injection. In pre-clinical studies, we have demonstrated that after a brief period of culture, EPCs develop a strong angiogenic phenotype and are efficiently filtered by the lung, lodging at the level of the pre-capillary arteriole, where these cells can contribute to local lung microvascular repair to restore the continuity of the pulmonary microcirculation.

Gene therapy offers the potential to address genetic abnormalities underlying PAH, and to produce a durable improvement or even reversal of the dysfunctional and damaged vessels in the pulmonary vasculature. Pre-clinical studies in experimental models of PAH have shown that cell-based gene transfer of human endothelial nitric oxide synthase (eNOS) is effective in preventing PAH induced by monocrotaline (MCT), which produces a rapidly progressive and universally fatal form of this disease in rats. As well, we have shown that eNOS gene therapy can reverse established PAH in this model, and that the combination of eNOS gene transfer with EPCs (i.e., cell and gene therapy) provided the greatest benefit in terms of reversing established PAH in animal models. These data are detailed in the Investigator's Brochure.

A phase 1 dose-ranging safety trial, Pulmonary Hypertension and Cell Therapy (PHACeT; ClinicalTrials.gov Identifier: NCT00469027), was performed using autologous EPC transiently transfected by electroporation with plasmid DNA containing the full coding sequence of human eNOS. This study enrolled 7 patients with a diagnosis of idiopathic PAH; 3 received a total of 7 million cells (Panel 1), 3 received 23 million cells (Panel 2) and one patient received 50 million cells (Panel 3). Cells were delivered by central venous injection through the pacing port of a Swan-Ganz catheter with continuous monitoring of pulmonary arterial pressures in an intensive care environment. For added safety, the total cell dose delivered was divided into 3 aliquots and delivered over 3 days such that the initial "test" dose in Panel 1 was 1 million cells on day 1, followed by 3 million on days 2 and 3 (total of 7 million cells). Participants in Panel 2 received 3, 10 and 10 million cells (total of 23 million cells), and those in Panel 3 received 10, 20 and 20 million cells (total of 50 million cells), on days 1, 2 and 3 respectively. The primary safety analysis was change in pulmonary hemodynamic parameters before and 30 minutes after cell delivery. No deterioration in pulmonary hemodynamics was seen during cell delivery. Administration of 20 million cells as a single injection repeated on two consecutive days in Panel 3 and 23 million cells as a cumulative cell dose delivered over 3 days in Panel 2 were well tolerated. Moreover, there was a statistical trend towards improved total pulmonary resistance over the 3 day delivery period; although no sustained reduction in pulmonary arterial pressure or resistance was seen at 3 months. Of note, there were significant improvements in 6 minute walk distance (6MWD), which peaked at 1 month (~60 meters, $p<0.01$), persisting to 6 months (~40 meters, $p<0.05$). Quality of life measures (SF-36) and functional class were improved over the course of the study as well. Only one patient died during the study period.

The SAPPHIRE trial includes two important design changes to increase the potential efficacy and improve the safety of eNOS gene-enhanced cell therapy for PAH. The first is to repeat cell therapy on a monthly basis, to increase the cumulative cell "dose" that will be delivered over the 12 month study period. Thus, participants will receive 4 or 8 monthly injections of 20 million cells (depending on the study arm), for a maximum of 80 or 160 million cells, respectively. The second is to use minicircle DNA, rather than conventional bacterial plasmid DNA, for eNOS transfection. The advantages of minicircle transfection include greater efficiency of transfection and expression of the gene product as well as to minimize exposure of cells to bacterial DNA sequences. Bacterial DNA contains cPG motifs which can induce an innate immune response leading to rapid clearance of the foreign DNA from the cell. In contrast, the minicircle technology removes most of the bacterial backbone of the plasmid and generates small rings of DNA that contains only the promoter and the human eNOS sequence. These advantages will increase the magnitude and duration of the eNOS transgene expression, while also reducing the chance of host immune response.

Objectives

SAPPHIRE seeks to establish the short and longer-term efficacy and safety of intravenous delivery of autologous culture-derived, eNOS minicircle transfected EPCs in participants with severe PAH.

Primary objective: To determine whether a course of four doses of eNOS gene-enhanced autologous EPCs, delivered monthly, is effective in improving 6 minute walk distance (6MWD) at 6 months in participants with severe PAH who are also receiving PAH-targeted treatments (Arms 2 and 3 vs. Arm 1; see Figure 1).

Secondary objectives:

1. To compare the sustained effect of delivery of 4 vs. 8 doses of the cell therapy product on 6MWD at 12 months (i.e. Arm 2 vs. Arm 3)
2. To assess durability of any improvement in 6MWD at 3 vs. 9 months post last dose of cell therapy product (Arm 2 only)
3. To determine the incremental effect of delivery of 8 vs. 4 doses of the cell therapy product on 6MWD at 6 and 12 months (Arm 3 only)
4. To assess the cumulative effect of 4 doses of the cell therapy product on pulmonary vascular resistance at 6 months (Arms 2 and 3 vs. Arm 1)
5. To compare the sustained effect of delivery of 4 vs. 8 doses of the cell therapy product on pulmonary vascular resistance at 12 months (i.e. Arm 2 vs. Arm 3)
6. To assess durability of any improvement in pulmonary vascular resistance at 3 vs. 9 months post last dose of cell therapy product (Arm 2 only)
7. To determine the incremental effect of delivery of 8 vs. 4 doses of the cell therapy product on pulmonary vascular resistance at 6 and 12 months (Arm 3 only)
8. To assess effect of cell therapy on Quality of Life measured by the SF-36 at 6 months (Arms 2 and 3 vs. Arm 1)
9. To compare the sustained effect of delivery of 4 vs. 8 doses of the cell therapy product on Quality of Life measured by the SF-36 at 12 months (i.e. Arm 2 vs. Arm 3)
10. To assess the effect of cell therapy on a combined endpoint of death, or clinical worsening of pulmonary arterial hypertension at 6 and 12 months
11. To assess effect of cell therapy on measures of RV function by echocardiography at 6 and 12 months
12. To assess effect of cell therapy on RV function by Magnetic Resonance Imaging (MRI) at sites where this is available in patients with no contraindications for MRI (i.e. prostacyclin infusion pump, claustrophobia) at 6 and 12 months

Section 2: Study Methods

2.1 Trial Design

SAPPHIRE will use autologous progenitor cell-based gene delivery to enhance lung microvascular repair and regeneration in severe symptomatic PAH. A total of 45 participants will be enrolled in this multi-centre, late phase, randomized, double-blind, placebo-controlled, 3-arm protocol. Up to nine centres across Canada will participate. The trial is sponsored by Northern Therapeutics and has been registered at www.ClinicalTrials.gov (NCT03001414). Study recruitment is expected to take place over a 5-year period.

The primary endpoint is the 6 minute walk distance (6MWD) at 6 months. The primary effect measure is difference in 6MWD at 6 months between patients receiving a course of 4 monthly doses of eNOS-EPCs compared to placebo. As well, a number of secondary endpoint measures will be performed to provide additional insights into the safety and efficacy of this treatment.

Study participants will be randomized into one of three arms (**Figure 1**):

Arm 1. Four monthly IV injections of the placebo saline solution (Plasma-Lyte A) in the first 6 months (Course 1) followed by four monthly IV injections of 20 million autologous eNOS-transfected EPCs in the second 6 months (Course 2; total cumulative dose of 80 million cells).

Arm 2. Four monthly IV injections of 20 million autologous eNOS-transfected EPCs in the first 6 months (Course 1) followed by four monthly IV injections of placebo (Plasma-Lyte A) in the second 6 months (Course 2; total cumulative dose of 80 million cells).

Arm 3. Four monthly IV injections of 20 million autologous eNOS-transfected EPCs in the first 6 months (Course 1) followed by a repeat of four monthly IV injections of 20 million eNOS-transfected autologous EPCs in the second 6 months (Course 2; total cumulative dose of 160 million cells).

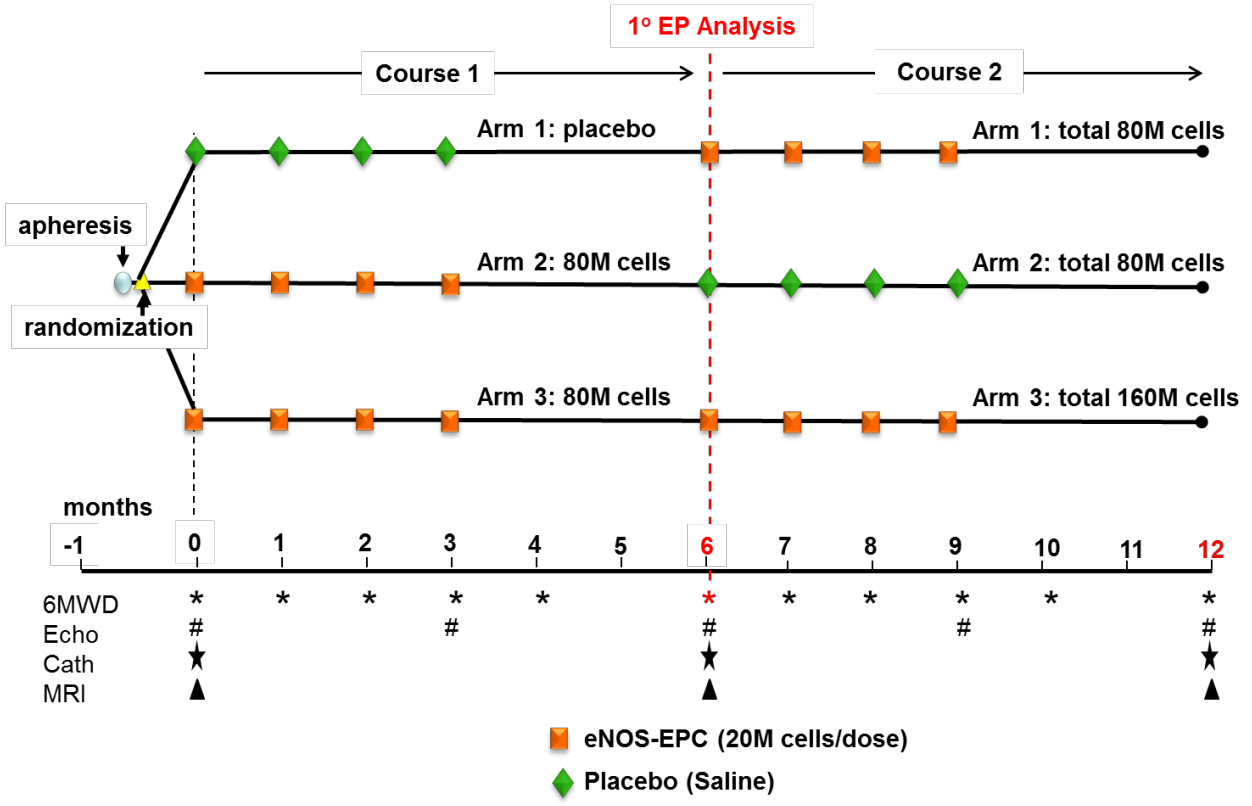


Figure 1: The SAPPHIRE 3-Arm trial design.

Rationale for the 3-arm trial design: The SAPPHIRE trial is designed to maximize the generation of relevant results to inform further development or application of the cell therapy approach. The rationale for the main elements of the study is summarized below.

- **Course 1:** The first 6 months of the study has a standard design with 2:1 randomization to study product or placebo:
  - Arm 1: Participants will receive 4 monthly placebo injections.
  - Arms 2 and 3: Participants will receive 4 monthly injections of the cell therapy product.

  The primary effect measure is at the 6 month time point.

  However, the study continues beyond 6 months in order to address a number of important secondary endpoints. ***Importantly, the blind will continue over the entire 12 month period of the study***.

- **Course 2**: In the second 6 month period, the study treatments will be altered to address specific questions about the timing and durability of this cell therapy:
  - Arm 1: Participants previously receiving placebo will now receive a course of 4 cell therapy treatments allowing all participants in this trial to have the opportunity to be given the active study product.

- Arm 2: Participants previously receiving EPCs will now receive the placebo study product to allow an assessment of the durability of any potential benefit of the cell therapy provided in Course 1 over the remaining 6 months of the study.
- Arm 3: Participants previously receiving EPCs will now receive a second course of cell therapy treatments, for a total of 8 doses of eNOS-transfected EPCs. This Arm will determine whether 8 repeated doses of the cell therapy product provides a greater incremental benefit than 4 doses.

After the 12-month treatment and follow-up period is complete, the study will be converted to a long-term follow-up registry extending to 10 years.

2.2 Randomization and Blinding

Randomization

Eligible participants will be randomized only following the completion of a satisfactory apheresis collection and receipt of the sample by the CMF. CMF personnel who have been trained by the sponsor to perform randomizations will access the SAPPHIRE web-based randomization system available from the Ottawa Methods Centre (OMC) located at OHRI. Personnel requiring access to this system will receive individual confidential login accounts from Data Management Services at the OMC.

After accessing the SAPPHIRE randomization system, the web-page will prompt the user to confirm participant eligibility by completing an electronic randomization case report form. Once completed, the web-based program will generate a randomization number on the screen. The requesting CMF user will print the screenshot containing the randomization number and will file it in the CMF participant binder. A copy will then be scanned and emailed to the study site for placement in the participant's study file.

Randomization to treatment allocation will be performed using a randomly permuted block design of fixed sizes of 3, stratified based on the average of the two screening 6MWD test results (≤ 300 m vs. > 300m) and type of PAH (scleroderma associated PAH vs. all other eligible PAH types). Since our sample size for this late phase trial is relatively small, stratification is used to promote balance across the arms with respect to the baseline measure of the primary outcome, as well as type of PAH, an important prognostic factor for response to therapy. In particular, scleroderma associated PAH tends to progress more rapidly and show less favourable response to PAH-specific therapies. The allocation sequence will be generated using a computer-generated algorithm operated by the unblinded statistician for the trial located at the OMC. The study sponsor/CRO will monitor all randomizations throughout the conduct of the trial. The OMC will also generate a blinded email notification to the study site, study sponsor, and the CMF as confirmation of successful randomization. The CMF will subsequently receive a notification with the unblinded treatment allocation in order to proceed with manufacturing of the participant's study product.

Blinding

Treatment allocation will remain blinded to all study participants, investigators, clinical research staff, persons performing study assessments/procedures, and data analysts from the time of randomization until database lock. Only the following study personnel will have access to unblinded treatment allocation: the unblinded study statistician and CMF personnel responsible for study product manufacturing. The study statistician will be the author of the Master Randomization List and will act as the primary contact to the CMF for any questions pertaining to study treatment allocation. The total processing period in the CMF will be equal (7±2 days) for all study products, including placebo, in order to maintain blinding of the investigator and clinical research staff. The OMC study statistician will securely maintain the confidentiality of the Master Randomization List from all other parties involved in the trial prior to database lock.

Emergency Unblinding

Since there is no antidote to cell therapy, the need for emergency unblinding during the 12 month treatment/follow-up period will be unlikely; however, unblinding may be undertaken if it is thought to be essential for effective treatment and management decisions for the study participant. The site Investigator will be responsible for contacting the trial medical consultant, Dr. Duncan J. Stewart, directly to discuss the rationale for unblinding. After consultation with Dr. Stewart (or delegate), if there is a need to unblind, Dr. Stewart will then contact the Manager of Data Management Services at the Ottawa Methods Centre (OMC) to ascertain the actual treatment assignment. The OMC is available 24 hours/day, 7 days/week. Dr. Stewart will then relay the treatment assignment to the site Investigator. The participant will then no longer receive any further planned administrations of study product but will be followed for the duration of their study period as an “off-protocol” study participant. Study personnel will record and report the unblinding as a protocol violation. Protocol violations related to unblinding will be monitored by the Study Executive and DSMB. Local personnel in the cell manufacturing facility (CMF) will also have a record of the participant’s treatment allocation.

2.3 Sample Size

A total sample size of 39 participants (13 per arm) in an analysis of covariance using a planned comparison between the average of the two treatment arms versus control achieves 80% power to detect a minimally important mean difference of **50 meters** in the primary outcome (6MWD) using an F-test with a two-sided 5% significance level. The SAS procedure GLMPOWER was used to conduct the calculation (SAS Institute Inc. 2008. SAS/STAT® 9.2. User’s Guide. Cary, NC: SAS Institute Inc). The common standard deviation is assumed to be 85 and the correlation between baseline and 6 months is 0.80. These estimates are similar to those observed in our PHACeT trial where the standard deviation was 80 meters and the correlation between baseline and 6 month was 0.86. To account for 10% potential attrition, we will enroll and randomize 45 subjects (15 allocated to each arm).

2.4 Framework

This will be a superiority trial, with primary aim to determine whether receiving a course of 4 monthly doses of eNOS-EPCs will improve the 6 minute walk distance (6MWD) at 6 months

compared to placebo. A minimally important mean difference of 50 meters was considered for this hypothesis.

2.5 Statistical Interim analyses and stopping guidance

Interim Analysis: No formal interim analysis will be conducted for efficacy. A safety analysis is planned after the first twelve participants complete primary endpoint data collection at the 6-month follow-up visit. Safety data will be sorted by treatment arms (blinded) to determine the distribution of potential safety concerns and be provided to the DSMB for review. The unblinded treatment allocation will be available from the statistician should the DSMB determine that a safety concern exists that necessitates unblinding.

Study Stopping Criteria: Enrolment and administration of the study product at all sites will be immediately placed on hold with the occurrence of any one of the following: i) death, ii) major cardiovascular event, or iii) a life-threatening response (i.e. immune reaction) that is deemed "*definitely related*" to the *study product* by the trial Data Safety and Monitoring Board (DSMB). The Chair of the DSMB will be notified promptly and be provided with copies of de-identified source information for review and will make recommendations for protocol amendments or revisions to the Investigator's Brochure and/or the risk section of the informed consent form as required. The sponsor will inform Health Canada immediately of the "*definitely-related*" *unexpected and serious event* that met the predefined stopping criterion. Site investigators will be provided with the case information for reporting to their local REB. The Steering Committee may consider resuming the trial after consideration of the DSMB recommendations, and, if applicable, any submission and approval of any suggested protocol/consent amendments by Health Canada and the local REBs.

Premature Trial Discontinuation: Premature trial discontinuation may occur as a result of an event that triggers the protocol-defined study stopping criteria. Termination or suspension of the trial may also result from a recommendation made by the Data and Safety Monitoring Board. The Steering Committee will review any DSMB recommendations and will rule on any consequential actions to be implemented by the sponsor. If the sponsor is required to terminate or suspend the trial, the sponsor will notify the principal investigators in writing. The investigators, in turn will promptly inform their research staff, the trial participants and their local Research Ethics Board of this development. The sponsor will follow-up with the investigative sites by sending the principal investigators written instructions for participant and trial close-out procedures. The investigators in turn, should assure appropriate follow-up for the study participants. The sponsor will notify the Biologics and Genetic Therapies Directorate of Health Canada no later than 15 calendar days after the date of trial discontinuation.

The sponsor may terminate a site's participation due lack of enrollment, concerns of data integrity, protocol noncompliance or investigator conflict of interest. If the investigator terminates or suspends the trial without prior agreement of the sponsor, the investigator will inform the institution where applicable, and the investigator/institution must promptly inform the sponsor and the REB and should provide both with a detailed written explanation of the termination or suspension.

If the Research Ethics Board (REB) terminates or suspends approval of a trial, the principal investigator will inform the institution where applicable and the investigator/institution must

promptly notify the sponsor and provide the sponsor with a detailed written explanation of the termination or suspension.

2.6 Timing of final analysis

Final analyses will be conducted following the end of the trial, once 45 participants are randomized and complete the 12-month treatment phase of the trial. The data will be collected, cleaned, verified, and locked by the OHRI Methods Centre data management services (DMS) group. Final analysis will be commenced once the final lock has been confirmed by the Trial Medical Consultant or the Clinical Trial Manager. The datasets will be provided to the trial statistician who will prepare reports and models for the analysis of the trial.

2.7 Timing of outcome assessment

Appendix B – Schedule of Assessments

Time point / **Assessment**	**Screening phase**	**Treatment Phase**								**Registry**
		Month 0	**Months 1, 2, 3 (±4 days)**	**Month 4 (±4 days)**	**Month 6 (±5 days)**	**Months 7, 8, 9 (±4 days)**	**Month 10 (±4 days)**	**Month 12 (±5 days)**	**Premature Withdrawal** *(see Note[11])*	**Yearly Telephone Call x 10 (±15 days)**
Informed consent	X									
Eligibility Assessment	X									
Demographics, medical history	X									
Concomitant Medication Recording	X	X	X	X	X	X	X	X	X	
Physical exam by investigator	X	X	X	X	X	X	X	X	X	
WHO class	X	X	X	X	X	X	X	X	X	
Vitals signs (*See Note[1]*)	X	X	X	X	X	X	X	X	X	
Body weight & height	X									
O_2 saturation	X	X	X	X	X	X	X	X	X	

Clinical Blood Tests (*See Note[2]*)	**X(a)**	**X(b)**	**X(c)**	**X(c)**	**X(b)**	**X(c)**	**X(c)**	**X(b)**	**X(b)**	
Inflammatory biomarkers (*See Note[3]*)		**X**	**X**	**X**	**X**	**X**	**X**	**X**		
Biomarkers for storage (*See Note[4]*)		**X**			**X**			**X**		
Urine pregnancy test (*See Note[5]*)	**X[1]**	**X[2]**	**X[2]**	**X**	**X[2]**	**X[2]**	**X**	**X**	**X**	
Urinalysis	**X**	**X**			**X**			**X**	**X**	
12-Lead ECG	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
6MWD Test (*See Note[6]*)	**X(*)**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Borg Dyspnea Index (*See Note[7]*)	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Apheresis (*See Note[8]*)	**X**									
QOL Questionnaire (*See Note[9]*)		**X**	**X[3]**		**X**	**X[4]**		**X**		
Chest x-ray	**X**				**X**			**X**		
2D Echocardiogram with Doppler and saline contrast (See *Note[10]*)		**X**	**X[5]**		**X**	**X[6]**		**X**		
Cardiac MRI		**X**			**X**			**X**		
Hemodynamic Measurements		**X**			**X**			**X**		
PFTs		**X**			**X**			**X**		
Study Product Administration		**X**	**X**		**X**	**X**				
A/E & SAE Assessment		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

NOTE[1]: **Vital Signs:** Blood pressure, heart rate, respirations (all measured seated after 10 minutes of rest)

NOTE[2]: Clinical Blood Tests:

X(a) sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR, HIV-1/2, HBV, HCV, HTLV-I/II, *Treponema pallidum* (TP) Antibodies, ß-hCG for females with childbearing potential

X(b) sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR, brain natriuretic peptide (BNP), C-reactive protein (CRP)

X(c) sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, BNP, CRP

NOTE[3]: Inflammatory Biomarkers: For treatment visits (Month 0-3, 6-9), samples must be drawn immediately prior to study product delivery.

NOTE[4]: Biomarkers for Storage: Samples will be stored in the OHRI biorespository for up to 15 years.

NOTE[5]: Urine Pregnancy Test: **X^1** Must be performed in the morning prior to apheresis on the same day

X^2 Must be performed prior to product administration on the same day

NOTE[6]: 6MWD Test: **X(*)** During screening, two 6MWD tests to be performed within 24 hours with at least 1 hour of rest between the tests.

X One 6MWD test to be performed at all other visits

NOTE[7]: Borg Dyspnea Index: Measured at the beginning and end of the 6MWD test

NOTE[8]: Apheresis: To be performed within 3 months of completion of screening assessments.

NOTE[9]: QOL Questionnaire: **X^3** Administered only at 3 month visit

X^4 Administered only at 9 month visit

NOTE[10]: 2D Echocardiogram: **X^5** Performed only at 3 month visit

X^6 Performed only at 9 month visit

NOTE[11]: Premature Withdrawal Visit: Performed for participants withdrawn from the trial during the treatment phase:

1. 30 ± 2 days after the last study product injection (for participants who exit the treatment phase between Months 0-4 or 6-10)
2. 5 ± 2 days following withdrawal from the treatment phase (for participants who exit the treatment phase between Months 4-6 or 10-12)

Section 3: Statistical Principles

3.1 Confidence Intervals and P-values

Statistical significance will be set at α=0.05. There is a single primary outcome. Prespecified secondary outcomes and subgroup analyses will be performed without adjustment for multiplicity. We will report measures of effect with 95% individual confidence intervals.

3.2 Adherence and protocol deviations

Adherence to the intervention is defined as study participants receiving the study product according to their allocated treatment arm for 75% of the study product deliveries during the first course of treatment (minimum of 3 deliveries during the first 6 months) and 75% of the study product deliveries during the second course of treatment (minimum of 3 deliveries during the second 6 months).

Deviations that my impact the analysis include:

1. Ineligible patients may be mistakenly randomized into the trial (randomization errors). While full source verification for participant eligibility is performed in accordance with the monitoring plan for the trial, the initial monitoring visit is scheduled to take place only after a participant has been randomized. Deviations arising from randomization errors will result in participant exclusion from the analysis population.
2. Patients may be incorrectly stratified during randomization (according to their 6MWD and type of PAH). If such cases arise, the participants will still be included in the analysis population.

All deviations will be monitored and recorded in the trial electronic data capture system (EDCS) on an on-going basis for the duration of the trial. The study sponsor will be immediately notified by the study monitors of any errors in randomizing ineligible participants, and the data will subsequently be entered into the protocol deviation eCRF to document the reason for the deviation along with the actions taken.

3.3 Analysis populations

The primary analyses will be by intent-to-treat but we will also conduct “per protocol" and "as treated" analyses.

Intent-to-treat analysis will include all randomized participants, including those who have not received a dose of the study product. Analysis will be based on the initial treatment assignment.

Per protocol analysis will include all randomized participants who have received at least 3 doses of the allocated study product during the first course of treatment (first 6 months), and at least 3 doses of the allocated study product during the second course of treatment (second 6 months), with all primary outcome measures having been captured.

As treated analysis will include all randomized participants who have received at least 1 dose of the study product. Analysis will be based on the actual treatment received rather than on the initial treatment assignment.

Section 4 – Trial Population

4.1 Eligibility

Referrals for screening will be made by physicians who follow patients for clinical management of PAH at participating SAPPHIRE trial sites, all of which are specialized pulmonary hypertension clinics. Health records will be reviewed in compliance with the Federal Personal Information Protection and Electronic Documents Act (PIPEDA), provincial privacy laws, and local hospital privacy policies.

The research consent process will be initiated at the research site for potential participants who appear to be eligible based on available clinical information. For potential participants who wish to proceed with the trial and who provide written consent, research investigations will then commence. Screening investigations will be completed as outlined in the schedule of assessments in **Appendix B**.

Inclusion Criteria:

Patients participating in the study must meet ALL of the following inclusion criteria:

- Age ≥ 18 years, ≤ 80 years
- Established diagnosis of PAH due to the following:
    - Idiopathic or heritable PAH;
    - Scleroderma associated PAH (limited or diffuse);
    - Drugs (anorexigens) or toxins;
    - Congenital heart defects (atrial septal defects, ventricular septal defects, and patent ductus arteriosus) repaired ≥ 1 years
- WHO functional class II, III, or IV on appropriate stable therapy for PAH for at least 3 months prior to the screening period and up until randomization, apart from modification of anticoagulant or diuretic dosages, or small adjustments in prostaglandin dose that are considered by the Investigator to be consistent with stable parenteral therapy.
- Able to walk unassisted (oxygen use allowed). Aids for carrying oxygen (such as a wheel chair or walker) are permitted provided they are not also required as mobility aids.
- An average 6-Minute Walk Distance (6MWD) of ≥ 125 meters and ≤ 440 meters on two consecutive tests during the Screening period
- Previous diagnostic right heart cardiac catheterization (RHC) at the time of PAH diagnosis with findings consistent with PAH: specifically, mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg (at rest); pulmonary capillary wedge pressure (PCWP) (or left ventricular end diastolic pressure) ≤15 mmHg, and pulmonary vascular resistance (PVR) >5 WU. If repeat testing has occurred since initial diagnosis, the most recent results should be used.
- Echocardiography performed within 12 months prior to the Screening Period confirming a left atrial volume index (LAVI) of ≤ 34 ml/m^2 and the absence of any clinically significant left heart disease including evidence of more than mild left-sided valvular heart disease, systolic or diastolic left ventricular dysfunction

- Ventilation and perfusion (VQ) nuclear scan performed as part of the initial workup to establish the diagnosis of PAH showing absence (i.e. low probability) of pulmonary embolism. If repeat testing has occurred since initial diagnosis, the most recent results should be used.
- Pulmonary function tests conducted within 2 years prior to the Screening Period to confirm: total lung capacity (TLC) ≥ 65% the predicted value; and forced expiratory volume at one second (FEV1) of ≥ 65% the predicted value
- Must have a resting arterial oxygen saturation (SaO2) ≥88% with or without supplemental oxygen as measured by pulse oximetry at the Screening Visit
- Must not be enrolled in an exercise training program for pulmonary rehabilitation within 3 months prior to the Screening Visit and must agree not to enroll in an exercise training program for pulmonary rehabilitation during the Screening Period and the first 6 months of the study. Participants enrolled in an exercise program for pulmonary rehabilitation 3 months prior to screening may enter the study if they agree to maintain their current level of rehabilitation for the first 6 months of the study
- Women of child-bearing potential (defined as less than 1 year post-menopausal and not surgically sterile) must be practicing abstinence or using two highly effective methods of contraception (defined as a method of birth control that results in a low failure rate, i.e., less than 1% per year, such as approved hormonal contraceptives, barrier methods [such as a condom or diaphragm] used with a spermicide, or an intrauterine device). Subjects must have a negative ß-hCG pregnancy test during the Screening period and negative urine pregnancy test results at all other study visits
- Must be willing and able to comply with study requirements and restrictions.

Exclusion Criteria*:*

Patients will not be included in the study if ANY of the following exclusion criteria are present:

- Pregnant or lactating
- PAH related to any condition not covered under inclusion criteria, including but not limited to pulmonary venous hypertension, pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis, or chronic thromboembolic pulmonary hypertension
- Evidence of more than mild interstitial lung disease on Chest CT within the last 5 years (last 3 years for patients with scleroderma associated PAH)
- Treatment with an investigational drug, device or therapy within 3 months prior to the screening period or is scheduled to receive an investigational drug, device or therapy during the course of the study
- Any musculoskeletal disease or any other disease that would significantly limit ambulation
- Unrepaired or recently repaired (< 1 year) congenital systemic-to-pulmonary shunt other than patent foramen ovale
- Patients having three or more of the following four AMBITION study[1] HFpEF risk factors will be excluded:

[1] Galiè N, Barberà JA, Frost AE, et al. Initial use of ambrisentan plus tadalafil in pulmonary arterial hypertension. N Engl J Med 2015;373:834-44. DOI: 10.1056/NEJMoa1413687

  - BMI ≥ 30 kg/m^2,
  - History of essential hypertension,
  - Diabetes mellitus (any type)
  - Historical evidence of significant coronary artery disease (CAD) by ANY ONE of the following:
    - History of MI
    - History of PCI
    - Prior coronary angiography evidence of CAD (>50% stenosis in ≥1 vessel)
    - Previous positive Stress Test
    - Previous CABG
    - Stable angina
- Creatinine clearance <30 ml/min (using the Cockroft-Gault formula) or requires hemodialysis
- Inability to undergo the apheresis procedure due to poor venous access or laboratory tests that are not within acceptable ranges (not including INR for patients on Coumadin)
- Childs-Pugh class C liver cirrhosis
- Previous atrial septostomy
- Any other clinically significant illness or abnormal laboratory values (measured during the screening period) that, in the opinion of the Investigator, might put the subject at risk of harm during the study or might adversely affect the interpretation of the study data
- Anticipated survival less than 1 year due to concomitant disease
- History of cancer in the past 5 years (except for low grade and fully resolved non-melanoma skin cancer)
- Results during screening consistent with current infection with HIV, Hepatitis B(HBV) or C(HCV), human T-cell lymphotropic virus (HTLV- I/II) or syphilis
- Systemic arterial systolic blood pressure < 85 mm Hg
- Known allergy to gentamicin or amphotericin
- Patients who have participated in any gene therapy study or an angiogenic growth factor protein study
- Patients unable to provide informed consent and comply with the visit schedule.

Screening Evaluations:

After informed consent has been obtained and documented, participants will be screened for study-specific inclusion/exclusion criteria through screening visit assessments and procedures, which will occur within a 2 week period, no more than 3 months prior to the apheresis procedure and randomization. A detailed schedule of study assessments is found in **Appendix B**.

Each participant enrolled in the trial will be assigned a unique study number that will be used in all study-related documentation pertaining to the individual. The number will be a combination of the site number provided by the coordinating centre and a sequential enrolment number. In the event of a screen failure, a 2nd screening visit may be performed; however, a new study number must be assigned to the participant.

Screening evaluations will include the following:

- Recording of patient demographics, medical history, concomitant medications
- Physical examination by the principal investigator or a sub-investigator
- Vital signs (blood pressure, heart rate, respirations) and measurement of height and body weight
- Assessment and recording of WHO functional class *(*see **Appendix C**)
- Chest x-ray
- Resting 12 lead ECG
- O_2 saturation measurement by peripheral device
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, Creatinine, Calcium, Magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR, HIV-1/2, HBV, HCV, HTLV-I/II, *Treponema pallidum* (TP) Antibodies, ß-hCG for females with childbearing potential
- Urine collection for routine and microscopic analysis
- 6-minute walk distance (6MWD) test and Borg dyspnea index (two 6MWD tests to be performed within 24 hours, not less than 1 hour apart, with ≤ 15% variation) (see **Appendix D and E**)

Consenting participants who meet all eligibility criteria will undergo an apheresis procedure which will be performed at the participating site according to the SAPPHIRE Apheresis protocol (**Appendix F)**. Participants will then be randomized to one of three study groups (Figure 1) following a satisfactory cell collection procedure and receipt of the sample by the Cell Manufacturing Facility (CMF). In the case of an unsuccessful apheresis, the participant would be given the option of undergoing a second apheresis procedure which should be scheduled within the 3-month screening period to avoid having to repeat screening procedures.

4.2 Withdrawal/Follow-up

Premature Participant Withdrawal

Premature withdrawal of study participants may be based on any of the following reasons:

- Sponsor Recommendations (i.e. Safety-specific protocol requirements)
- Investigator Decision (i.e. Participant non-compliance or if participant cannot safely perform the procedures required in the protocol)
- Participant Decision (No longer wishes to take part in the trial)
- Death of the study participant
- Pregnancy during the 12 month study treatment/follow-up period (refer to Section 9.7 for guidance)

Following the receipt of the study product, a participant may withdraw consent for study follow-up at any time for any reason. In addition, a participant may be considered withdrawn for failure to return for study visits or become lost to follow up for any other reason.

If a participant withdraws consent for further study follow-up procedures and assessments, every effort should be made to continue to follow the participant for their safety and the collection of safety data. This would ideally include completion of a treatment phase premature withdrawal visit (see Treatment Phase Premature Withdrawal Visit) and the long-term follow-up registry annual phone calls.

The PI or research staff must determine whether the participant wishes to:

- Withdraw from study follow-up procedures and assessments, but permit telephone follow-up or review of clinic/hospital records for data collection
- Withdraw from study follow-up procedures and assessments and decline further contact, however permit their accumulated data to be included in the study analysis
- Withdraw from study follow-up procedures and assessments and decline further contact and access to personal health information and indicate data collected to date cannot be used in the final analysis. Source documents cannot be destroyed until completion of the long-term storage period (25 years).

The primary reason for premature withdrawal and the participant's decision to decline study follow-up must be recorded in the participant's source documents and on the Treatment Phase Exit eCRF.

Lost to Follow-up

In the case of a participant becoming lost to follow up, the investigator should demonstrate "due diligence" by performing the following recommended steps:

- Contact participant three times and clearly document those efforts. Contacts may be done either by telephone contacts, email or mail correspondence. Documentation of any telephone contacts should also include dates and times
- If unsuccessful after three times, a registered letter should be sent to the participant's address requesting the participant to sign for the letter to attempt to confirm survival status
- If no response, clearly document efforts and complete all source documents and eCRFs as applicable
- Confirm status as lost to follow-up.

Treatment Phase Premature Withdrawal Visit

In the event of early withdrawal from the 12-month treatment phase of the trial, a Premature Withdrawal visit will be completed with the following timing:

1. 30 ± 2 days after the last study product injection (for participants who exit the treatment phase between Months 0-4 or 6-10)
2. 5 ± 2 days following withdrawal from the treatment phase (for participants who exit the treatment phase between Months 4-6 or 10-12)

The following assessments will be performed:

- Physical exam by the principal investigator or co-investigator
- Concomitant medication recording
- Vital signs (blood pressure, heart rate, respirations)
- Assessment and recording of WHO functional class *(*see Appendix C)
- O_2 saturation (peripheral device)
- Laboratory tests: sodium, potassium, chloride, TCO_2, blood sugar, BUN, creatinine, calcium, magnesium, phosphorus, alkaline phosphatase, total bilirubin, CPK, troponin, AST (SGOT), ALT (SGPT), LDH, gamma GT, albumin, uric acid, serum ferritin, CBC with differential, ESR, PT, PTT, INR, brain natriuretic peptide (BNP), C-reactive protein (CRP)
- Urine collection for routine and microscopic analysis
- Urine pregnancy test for females with childbearing potential
- 6MWD test and Borg dyspnea index (see Appendix D and E)
- Resting 12 lead ECG
- Assessment of adverse events and serious adverse events

4.3 Baseline Patient Characteristics

Baseline patient characteristics will include the following variables:

- Age
- Gender
- Race
- PAH Diagnosis (Idiopathic/Hereditable, Scleroderma, Drugs/Toxins, Congenital Heart Defects)
- WHO Functional Class (at Month 0)
- Oxygen use
- Type of PAH medication (s)
- Number of PAH medications
- Oxygen Saturation (at Month 0)
- 6MWD (at Month 0)
- RHC Results (at Month 0)
    - mPAP
    - PCWP
    - CO
    - PVR
- PFT results (at Month 0)
- Medical Conditions

Section 5 – Analysis

5.1 Outcome Definition

Outline and explain all primary and secondary outcomes. Include the specification of outcomes as they relate to timing and list the order in which primary or key secondary end points will be tested.

Primary Outcome

The primary outcome of this trial is 6-minute walk distance (6MWD) at 6 months.

Secondary Outcomes

Secondary outcomes include 6-minute walk distance (6MWD) at 3, 9 and 12 months, pulmonary hemodynamics (i.e., PAP, cardiac output, RAP PAWP) at 3, 6, 9 and 12 months, Quality of Life measured by the SF-36 at 6 and 12 months, combined endpoint of death, or clinical worsening of pulmonary arterial hypertension at 6 and 12 months and measures of RV function by echocardiography at 6 and 12 months.

Clinical worsening of pulmonary arterial hypertension will be defined by any of the following:

1. All-cause mortality
2. Hospitalization due to worsening cardiopulmonary status attributable to progression of disease
3. Decrease in 6MWD by ≥ 15%
4. Worsening of WHO functional class.

5.2 Analysis Methods

Descriptive Statistics

Descriptive statistics will be used to compare demographic characteristics and baseline prognostic factors of participants allocated to the three study arms. Measures of central tendency and dispersion will be calculated for all baseline variables in each study arm. Clinically important differences will be identified and adjusted for in all subsequent adjusted analyses. The normality of the distributions for primary and secondary outcome measures on a continuous scale will be assessed by visual inspection of histograms and normal probability plots. Normalizing transformations will be applied where necessary.

Analysis of Primary Outcome (6MWD at 6 months)

The primary analysis of our primary outcome, 6MWD assessed at baseline, one, two, three, four and six months, will use a general linear model with time (coded as a categorical variable) and group by time interaction as fixed covariates. As recommended for efficient analyses of longitudinal randomized controlled trials, baseline differences between the arms will be constrained through exclusion of the main effect for group[2]. In addition to providing the unadjusted effect, we will adjust our estimate for the stratification factors (6MWD ≤300m vs. > 300m and scleroderma APAH vs. all other eligible PAH categories), as well as age, gender and

[2] Fitzmaurice GM, Laird NM, Ware JH. Applied Longitudinal Analysis, Second Edition Hoboken, NJ: Wiley, 2011

baseline medication use (PDEVi and sGC stimulators vs. all other PAH-specific medications). The model will be estimated using Restricted Maximum Likelihood (REML), and degrees of freedom will be computed using the Kenward-Roger approach[3]. Suitable covariance structures to account for correlation among repeated measures over time will be identified using likelihood ratio tests and information criteria (AIC and BIC). For the primary endpoint, the adjusted least square mean difference between the average of the two active treatment arms (Arms 2 and 3) versus placebo (Arm 1) at 6 months, together with 95% confidence interval, will be obtained and assessed at the two-sided 5% level of significance. Additional exploratory pairwise comparisons of least square mean differences at one, two, three, and four months will be conducted.

Analysis of 6MWD at 12 months

To compare the effect of sustained delivery of 4 vs. 8 doses of the cell therapy product on 6MWD at 12 months (secondary objective 1), the regression model will include repeated measures up to 12 months and be used to estimate the adjusted least square mean difference between Arm 2 and Arm 3 at 12 months, together with 95% confidence interval. Additional pairwise comparisons of least square mean differences at 7, 8, 9 and 10 months will be conducted.

Analysis of the Durability of Primary Outcome (6MWD at 6 months)

To assess the durability of any improvement in 6MWD at 3 months vs. 9 months post last dose of cell therapy product (secondary objective 2), the repeated measures model up to month 12 will be used to estimate the least square mean difference from 6 months to 12 months within Arm 2 together with its 95% confidence interval, and used to test the hypothesis of no deterioration.

Analysis of the Incremental Effect of Delivery of 4 vs. 8 doses (6MWD from 6 to 12 months)

To estimate the incremental effect of delivery of 4 vs. 8 doses on the 6MWD (secondary objective 3), the repeated measures model up to month 12 will be used to estimate the least square mean difference from 6 months to 12 months within Arm 3 together with its 95% confidence interval.

Analysis of Additional Secondary Outcomes

Secondary objectives with respect to pulmonary hemodynamics (i.e., PAP, cardiac output, RAP PAWP), quality of life, and other continuous secondary outcomes will be analyzed using an approach similar to that used for analysis of our primary outcome of 6MWD. The combined endpoint of death or clinical worsening of pulmonary arterial hypertension up to 12 months will be described using Kaplan-Meier plots and compared across the 3 study arms using log-rank tests. Chi square test statistics and relative risks with 95% confidence intervals will be used to determine the effect of four doses of treatment on the combined endpoint at 6 months (Arm 1 vs. Arms 2 and 3). The combined endpoint at 12 months in Arm 3 will be described using 95% confidence interval and compared to historical event rates.

[3] Kenward MG, Roger JH. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics. 1997;53(3):983–97.

5.3 Missing Data

General linear models will be used to allow inclusion of all data in the analysis without the need to use ad hoc imputation procedures such as last observation carried forward. This maximum-likelihood based approach is fully efficient (assuming that covariates are completely observed).

Sensitivity Analyses

To assess for any potential bias due to attrition, we will examine differences between patients with complete data and those lost to follow-up based on all available covariates at baseline. A sensitivity analysis using multiple imputation under assumptions of "missing not at random" will be used to examine potential bias due to informative drop-out[4].

5.4 Harms

Adverse Event (AE)

An adverse event is any untoward medical occurrence in a research participant administered an investigational product and which does not necessarily have a causal relationship with the study product. An AE can therefore be any unfavourable and unintended sign (including an abnormal lab finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product.

The following definitions are to be used for guidance for determining severity of adverse events:

- Mild – An awareness of symptoms but easily tolerated
- Moderate – Symptoms interfere with normal daily activities
- Severe – Symptoms are incapacitating with inability to perform daily activities

Adverse Drug Reaction (ADR)

All noxious and unintended responses to an investigational product related to any dose should be considered adverse drug reactions. The phrase “responses to an investigational product” means that a causal relationship between the investigational product and an adverse event is at least a reasonable possibility (i.e. the relationship cannot be ruled out).

Serious Adverse Event (SAE) or Reaction

An SAE is any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect
- Based on medical judgement, is an important medical event that may require medical intervention to prevent one of the outcomes listed above.

[4] RJA Little. 1995. Modeling the drop-out mechanism in repeated-measures studies. Journal of the American Statistical Association 90(431): 1112-1121.

Note: The term "life-threatening" in the definition of "serious" refers to an event in when the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Unexpected Adverse Drug Reaction

UADR is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (i.e. the Investigator's Brochure for an unapproved investigational product). Reports which add significant information on specificity or severity of a known, already documented serious ADR constitute unexpected events. For example, an event more specific or more severe than described in the Investigator's Brochure would be considered "unexpected".

Expected Adverse Events in This Patient Population

The following is a list of expected adverse events in the PAH population which should be used for guidance when determining expectedness of adverse events for the trial:

- Right heart failure
    - Peripheral edema
    - Pleural or pericardial effusions
    - SOB/dyspnea
    - Liver dysfunction
- Chest pain, angina
- Syncope
- Hypotension
- Supraventricular arrhythmias, atrial fibrillation
- Side effects of concomitant PAH therapies
    - Prostanoids and prostaglandin receptor agonists: hypotension, headache, flushing, nausea, vomiting, diarrhea, jaw pain, thrombocytopenia, musculoskeletal pain
    - ERAs: peripheral edema, elevated LFTs, anemia, headache, nasal congestion
    - PDE5 inhibitors and sGC stimulators: Headache, flushing, hypotension, priapism, GERD, visual changes
    - High dose Lasix: Hearing loss
    - Anticoagulants: Bleeding, bruising
- Hypoxemia, intrapulmonary shunting
- Right to Left shunting with rising right sided pressures
- Renal dysfunction
- Pulmonary Edema (in the context of a very severe reduction in cardiac output)

Expected Adverse Events during Apheresis

Guidance for determining expectedness of adverse events that occur during apheresis can be found in the Apheresis Risks section of the Informed Consent Form.

Determination of Adverse Event Relationship to Study Product

For study participants having received at least one dose of the study product, guidance for determining the relationship and expectedness of adverse events can be found in Section 5 of the Investigator's Brochure.

Recording and Reporting Adverse Events

At each contact with the patient, the investigator will seek information on adverse events by specific questioning, and as indicated, by examination. All adverse events occurring during the study period will be recorded. Information on non-serious adverse events should be recorded in the case report form with indication of the possibility of relationship to the cell delivery procedure or the delivered cells. Causality of all adverse events must be determined by the Principal Investigator or a sub-investigator. **A serious adverse event form must be completed for all serious events and sent to Allphase Clinical Research Inc. by email to SapphireSAEReports@allphaseclinical.com within 24 hours of the investigator's knowledge of the event.**

At the time of the initial SAE report, the following must be recorded on the SAE Report Form: participant unique study number, randomization number, description of the event, date of onset of event, any treatment given for the event, whether investigative treatment was discontinued prematurely, the reason why the event was classified as serious, and the investigator's assessment of the association between the event and the study product/procedure. The Principal Investigator must also provide prompt reporting of any follow-up information on each serious adverse event. This should include copies of the completed supplemental serious adverse event forms and any other diagnostic information that would assist in the understanding of the event.

In addition, the Principal Investigator is required to report all adverse events to the REB in accordance with local reporting requirements. The following AEs do not normally require reporting to the REB:

- Serious adverse events that are considered expected
- Serious adverse events that are considered not related to the investigational product or research procedures, whether the event is expected or not
- Non-serious adverse events, whether expected or not.

Northern Therapeutics, Inc. is responsible for reporting *Unanticipated Problems (serious and unexpected events which are deemed related or possibly related)* to Health Canada. These events must be reported within **15 days** after becoming aware of the information if the event is neither fatal nor life threatening. However, if an event is fatal or life threatening, the information should be reported within **7 days** after becoming aware of the information. A complete report which includes an assessment of the importance and implication of any findings should be submitted within **8 days** of the initial report.

Analysis of Safety

Safety endpoints related to the tolerability and safety of injection of the study product in patients with severe PAH will be divided into early and late endpoints.

Early (within 1 hour of each study product administration):

- Clinical evidence of allergic or other immune reaction that in the opinion of the investigator is possibly related to the cell product injection
- Evidence of pulmonary or systemic embolization
- Symptomatic hypotension
- A clinically significant drop in O_2 saturation.

Late (within 1 month [visits 0-2 and 6-8] or 3 months [visits 3 and 9] of study product delivery):

- Evidence of significant immune activation (i.e. increases in ESR, CRP)
- Evidence of new and more than mild right to left shunting (i.e. greater than grade 2 shunting) by semi-quantitative analysis on agitated saline contrast echocardiography; or new evidence of rapid appearance of contrast in the left atrium.

A safety analysis is planned after the first twelve participants complete primary endpoint data collection at the 6-month follow-up visit. Safety data will be sorted by treatment arms (blinded) to determine the distribution of potential safety concerns and be provided to the DSMB for review. The unblinded treatment allocation will be available from the statistician should the DSMB determine that a safety concern exists that necessitates unblinding.

5.5 Data Management

Data will be collected at each participating site and entered into electronic CRFs (eCRFs) following scheduled assessment periods. The site coordinator will review the information entered into the eCRFs for completeness of information prior to submitting the forms to the OHRI Methods Centre data management services (DMS) group, the centralized data center where the data will be stored in an electronic database on a secure server with nightly backup. When queries arise, sites will be contacted to provide appropriate corrections. As part of quality assurance methods, all queries and corrections will be performed using electronic media to provide appropriate audit trails on the data elements that are changed. The Ottawa Methods Centre manager will oversee all activities at the data center, to prepare the interim and final datasets. The datasets will be provided to a Methods Centre statistician who will prepare reports and models for the analysis of the trial.

5.6 Programming Plans

Data manipulations and statistical analyses will be performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).